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Antifungal Activities of Curry Leaf (*Murraya Koengii)* Extract on Some Selected Fungi

Gabriel Charles Disegha[1] Vincent Onuegbu Izionworu [2*]

1. Faculty of Biological Sciences, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
2. Faculty of Engineering, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

* E-mail of the corresponding author: izy_vin@yahoo.co.uk or vincent.izionworu@ust.edu.ng

Abstract

Studies on the antifungal activity of aqueous and organic extracts of *Murraya koeingii* (curry leaf) on fungi was undertaken using *Candida albicans, Penicillium funiculosum, Penicillium camemberti, Aspergillus niger* as test organisms. Aqueous and organic extracts of the plants were obtained using standard techniques. The extracts were Cold aqueous extract (CAE), hot aqueous extract (HAE) and Ethanol extract (EE). Sensitivity test was carried out using Agar Well Diffusion Method; the set up was incubated at 37^0c for 24 hours. Development of zones of inhibition was observed and measured. This showed that some of the tested extracts demonstrated antifungal activities against the test organisms. Comparison of the extracts showed that Ethanol aqueous extract (EE) of *Murraya koengii* had high activity against all test organisms. The Minimum Inhibitory Concentration of Cold, Hot and Ethanol extracts of *Murraya koenigii* was 20g/ml for all the test organisms.Since the Cold and Hot extracts did not demonstrate high activity on the test organism, it could be concluded that the activity demonstrated by both Cold and Hot aqueous extract was due to the effect of the extracts. These results indicate that *Murraya koengii* can be used as an antifungal agent in the treatment of infections.

Keywords: antifungal, fungi, murraya koeingii (curry leaf), organisms, organic extract

1. Introduction

Natural products or plants derived compound contribute to a great extent in the fight against pathogenic microorganisms (Vyvyan, 2002). The biological inhibitions by different natural substances, such as essential oils and plant extracts have been investigated widely against fungi activities. Manohar *et al* (2001), analyzed *lippia jumelliana* (mold) against *Altaernaria solami, Schesotium cepivorium* and *collectotrichum coccodes*, similarly Singh *et al* (1998) determined fungi toxicity of extracts from 11 higher plants against a range of fungi based on sugarcane pathogens.

The World Health Organization (WHO) estimated that 80% of the populations of the developing countries rely on traditional medicines, mostly plant based drugs for their primary health care needs. During the last decade, demand for medicinal plants and its products as well as the traditional health system has attracted the world-wide interest due to the growing recognition of the drugs on natural products, food supplements and flavors (Dhar *et al*., 2000). In folk medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases, today a great number of different tea and other plant products are available in market including cosmetics and pharmaceuticals (Chopra, 1999).

The curry leaf also known as *Murraya koenigii* is tropical to India and Sri Lanka. It's leaves are used in many dishes and for medicinal purposes in India and neighboring countries (Bhattacharya ,1998), the leaves are generally called by the name "curry leaves" the curry leaves are also dried, powdered and stored. This can also be used in many dishes to enhance the taste and flavor.

The leaves of *Murraya Koenigii* are also used as a herb in Ayurvedic medicine. Their properties include much value as anti-diabetic, antioxidant, antimicrobial anti inflammatory (Maberly, 1998).

PLANT TAXONOMY

Kingdom	Plantae
Class	Eudicots
Order	Sapindales
Family	Ruter ceae
Genus	Murraya
Species	*M. Koenigii*
Botanical name	*Murraya Koenigii* (L)

The increasing threat of fungal infections to life, increase in the cost and allergic reactions produced by synthetic drugs, herbal medicine and to appreciate the gift of nature and better utilize the resources available in the tropical environment underscores the importance of this work.

Therefore. It is important to determine the antifungal activities of curry leave (*Murraya koenigii*) plant extracts using agar well diffusion method, investigating extract types *on Candida albicans, Penicillium camemberti, Penicillium funiculosum, Aspergillus niger* and to determine their efficacy by means of Minimum Inhibitory Concentration (MIC) of the extracts upon the test organisms.

2.0 Curry Leave

Curry leaf can be cultivated on red sandy, loam soils with good drainage are ideal for better leaf yield. The optimum temperature requirement is 26^0C to 37^0C (Gupta *et al.*, 1995). The main season of availability of curry leaf fruits is July to August. Within 3-4 days of collection of fruits, the seeds should be pulped and sown in nursery beds or poly bags. One year old seedlings are suitable for planting; one seedling is planted at the centre of the pit. Immediately after planting the pits are irrigated or the third day, the second irrigation is given once in a week (Handa, 1996).

Fresh curry leaves with stalks, can be stored in Zip lock bags and refrigerated for a maximum of 5 days after then they turn black and stinks. Curry leaves can also be stored by pat drying to make sure all water content is completely wiped off. The leaves are them spread over a paper towel and microwave for 1 minute (1000 watts) and then cooled. They are then stored in air-tight bottle which would stay for few months.

Fresh curry leaves with stalks removed are wiped stalks removed are wiped clean, lightly dry toasted and dried for dew days, this results in dry curry leaves. This stays for 6months if stored in air tight box.

The history of curry leaves dates back to the ancient period. The curry leave is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Later spread by Indian migrants, they are now grown in other areas of the world where Indian immigrants settled (Satyavathi *et al.*,1999).

The curry leafs are naturalized in forests and waste land throughout the Indian subcontinent except in the higher parts of the Himalayas. It is basically used as a spice and is an aromatic deciduous tree which is 5 meters tall and fifteen to forty centimeters in diameter. Curry powder made after grinding curry leaves, is used in the cooking of stew, and a variety of soups, chutneys, breakfast dishes like upma etc.

In some parts of Southeast Asia, curry leaves are chewed because they are believed to be beneficial to digestion and especially good for preventing diarrhea.

Curry leaves have been used for centuries almost in all the parts of the world. This herb has several medicinal properties. For instance, its leaves and bark can be used as a tonic, stomachic, stimulant and carminative (Kirtikar *et al.*, 2005*).* It can also help in reducing blood sugar if these leaves are consumed early in the morning in empty stomach. Curry leaves are also a good source of vitamin A and they provide a rich source of calcium.

Curry leaves are a great source of various vitamins and minerals. These include vitamin C, Vitamin A, folic acid, niacin, thiamin and riboflavin. Each of these vitamins plays an important role in development. Vitamin C is important for strengthening the immune system. Thiamin is known to have a role in organ and nervous system development. Other than vitamins and minerals, studies have shown that curry leaves contain antioxidants which are useful against free radical damage and oxidative stress in the body (Gupta *et al.*, 2009).A paste of curry leaves can be prepared against diseases like diarrhea and dysentery which often affect infants and toddlers (Satyawati *et al.*, 1999). Brian (2012) also identified that curry leaf extracts can assist the immune system and strengthen it.

Evergreen shrubs or small tree are up to 4mm tall. Leaves are arranged spirally, imparipinnate with 17-31 leaflets, stipules are absent. Leaflets are alternate, ovate to ovate lanceolate or orbicular, 2-5cm. Glandular are dotted, base obtuse to rounded and slightly asymmetrical, apex notched, margin is entire or irregularly toothed (Adewunmi, *et al*., 2001).

Flowers are bisexual, regular aromatic, pedicel is short, carlyx are with tiny ovate teeth, petals are oblanceolate to oblong, 5-7mm long, glandular, white. Stamens are 10 in number, ovary is superior, stigma capitates, fruit is ovoid to oblong, grandular berries. *Murraya koenigii* comprises about 15 species which are distributed from

continental Asia throughout the Malaysian region to north- eastern Australia and New Caledonia. Several species are cultivated throughout the tropics.

Curry leaves are propagated mainly from seeds must be ripe and fresh to plant, dried or shriveled fruits are hot viable. The whole fruit can be planted, but it's best to remove the pulp before planting in potting mix that is kept moist hot wet. Stem cutting can also be used for propagation. *Murraya Koenigii* grows best in deep well-drained soil in full sun to partial shade; in Africa it is either kept in large pots or grown in home garden (Akobundu, et al.,1998.) flowering is from March to June and fruiting from June to August. After transplanting it takes 12-15 months before the leaves can be harvested. Leaves can be refrigerated in airtight containers for up to 2 weeks without loss of flavor. They can also be frozen from storage for year round use.

2.1 Prospects

The essential oils and carbazole alkaloids from *murraya Koenigii* show many interesting pharmacological activities, including antibacterial, antioxidant, antitumor and hypoglycaemic activities, and more research is needed to evaluate its potential (Adebaye, 1997).
A large number of studies have been carried out on the chemical composition of the volatile oil of *Murraya Koenigii*. The leaves contain 35-65 compounds, mainly monoterpenes and sesquiterpenes depending on the seasonal variation, geographical location and age of the leaves, and which constitute about 95% of the essential oil (Onayade, *et al*., 2000).

Constituents of the essential oil of the leaves that can occur in high concentrations are α - copaene, β-ocimene and same minor compounds are β-pinene, β-phellandrene. *Murraya koenigii* is also a major source of carbozole alkaloids. From the stem bark murrayanine, girinimbine, isomurayazoline has been isolated (Adewunmi *et al*., 2001).

Many carbazole alkaloids exhibit significant antibacterial activity against a range of pathogenic bacteria. Leaf extracts also showed significant activity in the prevention and control of dental caries. Different extracts also exhibited significant antiflammatory, analgesic, antipyretic and antidiarrhoe, activities in studies using rats and mice. Many studies have been carried out on the use of the leaf extracted to treat diabetes.

Most studies confirm that leaf extracts lower the blood glucose levels in diabetic rats, although some tests are inconclusive. Different extracts of the leaves did not show any significant antihypertensive effects (Lawal *et al*., 2008). In an experimental setup, the leaves of *Murraya koenigii* significantly improved memory in mice thus exhibiting their potential in the management of Alzheimer's disease.

2.2 Uses Of *Murraya Koenigii*

A decoction of the leaves, bark and roots is taken throughout Asia as a febrifuge, tonic, stimulant and a stomachic. In the Indian Ocean Islands a leaf decoction is commonly drunk as an antihypertensive. The crushed bark and roots are used externally to treat skin eruptions and bites of poisonous animal's .The fresh leaves are eaten to treat dysentery and a leaf infusion is drunk to stop vomiting. In northern Nigeria *Murraya koenigii* is used traditionally as a stimulant and for management of diabetes (Palaniswamy,*et al*., 2003). The leaves are added top soup with crayfish to treat herpes, scurvy and post partum pain. Young twigs are used as tooth brush, and are reported to strengthen the gums and the teeth.

In Ayurvedic medicine preparations of the leaves, bark and roots are used for enhancing blood circulation, digestion and metabolism as well as for its anti-inflammatory actions (Onayade, *et al*., 2000).

The fresh or dried leaves are commonly used in flavoring vegetables but are reported to loose flavor upon drying. The essential oil (curry leaf oil), obtained from the leaves by distillation is used in the production of soap (Patel, *et al*., 2009).

In Africa trade of leaves for medicinal or culinary use is probably very limited, except for Mauritius and Reunion. In Asia the root wood is locally considered the best of all woods for making small objects. As the supplies are often extremely limited, the wood fetches high prices and is sold by the piece.

2.3 Herbs

Herbs are any plant used for flavoring, food, medicine or perfume. Herbs have a variety of uses including culinary, medicinal, and in some cases spiritual usage.

Accordingly to WHO, herbal medicines are described as finished labeled medicinal products that contain as ingredient aerial or underground parts of plant or other plant materials or combination thereof whether in a crude state or as plant preparations. Herbal medicines are considered cheap, affordable and readily available particularly in the rural areas where they are used as first treatment for sick people (Kirtikar, *et al*., 2006).

Herbal medicine is the study and the use of medicinal properties of plants (Lichterman, 2004). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals (Stepp, 2004).

Many of these plants have beneficial effects on long term health when consumed by humans, and can be used to effectively treat human diseases (Summer, 2000). Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines in 2001, researchers identified 122 compounds used in modern medicine which were derived from ethno medical plant sources. The use of herbs to treat disease is almost universal among non-industrialized societies and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care.

2.4 Antifungal Agents and Drugs

Antifungal drugs are medications used to treat mycoses such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as *cryptococcal meningitis* and others. There are different classes of antifungal drugs namely:- Polyene antifungal-drugs

A polyene is a molecule with multiple conjugated double bonds. A polyene antifungal is a macrocyclic polyene with a heavily hydroxylated region on the ring opposite the conjugated system. The polyene antimycotics bind with sterols in the fungal cell membrane principally ergosterol. As a result the cells contents leak and the cell dies polyene drugs include;-Nistatin, Pirtamicin, Amphotericin B. Other antifungal agents are imidazole, triazole and thiazole antifuyngals.

The azole agents have 5-membered organic rings that contain either two or three nitrogen molecules. The imidazole contain 2 nitrogen molecule and triazole contain 3 nitrogen molecule.

The clinically useful imidazole drugs are; clotrimazole, micronazole and kepoconazole. Two important triazoles are; Itraconazole and fluconazole. The triazole are fluconazole. The triazoles are newer agents and less toxic. Both groups inhibit the synthesis of ergosterol resulting in defective membrane that leak cytoplasmic content. The inhibition of ergosterol synthesis is on the enzyme cytochrome P450 14 & - dimethylase.

This enzyme converts lanosterol to ergosterol during synthesis and is required in fungi cell wall synthesis. These drugs may also block steroid synthesis in human. The types of imidazole antifungal drugs include: 1) **Ketoconazole:n** These are taken orally and also can be administered topically. It is used in treating systemic fungi infections due to *Histoplasma Capsulatum* and also used to treat other cutaneous mycoses. It's long term use in associated with side effects such as liver toxicity, nausea and vomiting. 2) **Miconazole**: These are drugs used topically to treat cutaneous infections such as athletic foot and vagina yeast infections. 3) **Clotrimazole**: It is used topically to treat cutaneous infections such as athletic foot and vagina yeast infections.

Types Of Triazole Antifungal Drugs include 1) **Fluconazole**: This can be administered either orally or intravenously. It is how routinely used to treat candidemia in non-neutropenic host and in treating cryptococcosis and coccidioides mycosis.

2) **Itraconazole**: This si taken orally and used in treating histoplasmosis, blastomycosis, coceidiodomycosis and other forms of Aspergillosis. It is less toxic than other systemic anti-fungal agents. Other antifungal drugs are: S-fluorocytosine/flucytosine, Griseofluvin, Toinaftate, Varriconazole, Caspofungin .

2.5 Development of Antifungal Drugs

Infectious diseases are as old as life itself. In the past 200 years, empirical science and serendipity have combined to bring us to our current state of knowledge (Ganz, 1985).

For example, Paul Ehirich is credited with the concept of selectivity, the postulated the existence of molecules that would bind to microbes, but not to the host cells. In the year 1877, Pasteur and Joubert reported how a culture should be inhibited by the products of a contaminating microorganism (Blondelle *et al.,* 2000).

The most celebrated of these observations is fleming's who in the year 1929 observed the lysis of the mold *penicillium notatum (*Fleming, 1929*).*

The development of antifungal drugs had lagged behind to those of antibacterial gents because of;

1) Bacteria are prokaryotic cells and have numerous structural and metabolic targets that differ from those of human host (Quellette, 1999).

2) Fungi are eukaryotes as human host, hence most agents toxic to fungi are also toxic to the host (Giglione, 2000).

3) Fungi generally grow slowly when cultivated on artificial media, and being in multicellular form are difficult to quantify than bacteria.

3.0 Materials and Methods

3.1 Materials

The work evaluated the antifungal activity of fresh curry leaf extract on some pathogenic fungi at different concentrations. The materials used for this project work include:

Media

The media used in this study is Sabouraud Dextrose Agar (65g) and Nutrient Broth (28g).

Apparatus and Equipment

Conical flasks, pipettes, beakers, Petri dishes, autoclave, incubators, test tubes, measuring cylinder, weighing balance, cotton wool, aluminum foil, spirit lamp, wire loop, glass spreader, masking tape, mortar and pestle, hand gloves, hot air oven, standard borer, and ring boiler. **Reagents**

Ethanol, distilled water, normal saline and hydrogen peroxide.

Samples

Fresh curry leaves, pure cultures of *Aspergillus niger, candida albicans, Penicillium camemberti and Penicillium funiculosum*.

3.2 Methods

Fresh leaves of *Murraya koenigii* was purchased from Mile3 market in Port Harcourt, in Rivers State and was brought to the Microbiology Department of Rivers State University of Science and Technology. It was washed thoroughly under running tap water. After washing the leaves were pounded in a mortar and then weighed into plastic containers using various grams. The soluble ingredients of the plant material were then extracted by solubilization in water (cold and hot) and ethanol as different solvents. The sample was identified and classified in the Department of Botany of Rivers State University of Science of Science and Technology. Pure cultures of Fungi specimens were collected from the Braithwaite memorial hospital in Rivers state and the Department of Microbiology in Rivers State University of Science and Technology. The glass ware used were washed with detergents, rinsed and sterilized in the hot air oven at 160^0c for 1hour before and after practical. All media used were also autoclaved at 121^0c for 15minutes at 15psi. Also wire loops, standard borers and the mouth of the flask and bottle containing agar and pure cultures were flamed before and after use. The fungi cultures were maintained at 4^0c in the refrigerator. These cultures were regularly sub cultured to fresh Sabouraud's Dextrose Agar slants every two weeks. 0.85g of sodium chloride (NaCl) was weighed using a weighing balance and dissolved in 100mls of distilled water. A 10ml pipette was used to transfer 9mls of the solution into sterilized test tubes and then autoclaved at 121^0c for 15minutes at 15psi (Cruickshank, *et al*., 1975).

65g of Sabouraud's Dextrose Agar (SDA) was weighed into 1000ml Erlenmeyer flask, distilled water (1000ml) was added gradually according to the manufacturer's instruction (Cruickshank *et al*., 1975). The mixture was homogenized with a stirrer and then flask was corked with cotton wool, aluminum foil and masking tape. The media was sterilized in the autoclave at 121^0c for15 minutes at 15psi. The sterilized media was subsequently allowed to cool to 45^0c and poured aseptically into Petri plates and allowed to solidify.

28g grams of nutrient broth was weighed into 500ml of distilled water and homogenized. A sterile pipette was used to pipette 9mls of the broth into various test tubes, corked and sterilized in an autoclave at 1210c for 15 minutes at 15psi.

Aqueous extraction of water soluble ingredients of plant material was carried out according to Bajwa *et al*., (2004). A 20%, 30% and 40% stock solution of plat extract was obtained by soaking the crushed plant materials in sterilized distilled water for 24 hours. It was then passed through Muslin cloth and finally through what man Filter paper No.1 under aseptic conditions.

Aqueous extraction of water soluble ingredients of plant material was carried out according to Bajwa *et al*., (2004). A 20%, 30%, 40% stock solution of plant extract was obtained by soaking the crushed plant materials in sterilized distilled hot water that was boiled to 100^0c for 24hours. It was then passed through muslin cloth and finally through what man's filter paper No.1 under aseptic conditions.

The ethanol extraction of the active ingredients was carried out according to Alkhail (2005). The test plant was crushed and extract of soluble ingredients was carried out by macerating 20%, 30% and 40% of crushed plant material in 100ml of ethanol for 24 hours. The extract was filtered by passing through what man's filter paper No.1. All extracts were stored at 4^0_C in pre sterilized containers to avoid contamination and prospective chemical alterations, the extracts were used within 3-4 days. To determine the minimum inhibitory concentration 13 test tubes were labeled and set up, then 1ml of broth was pipette into tubes 2-10, 11 and 13 after which 2ml broth was pipette into tube 12. Tube 11 is the inoculums control, tube 12 is the broth control and tube 13 is the dry control. 1ml of working drug (pure curry leaf) was pipette into tube 1, 2 and 12. A double dilution was prepared from tube 2 up to 10 using 1ml of the solution and 1ml of working inoculums (organism) was introduced into tubes 1 to 11. Tubes were incubated at 37^0c for 24 hours (Ochei *et al.*, 2000). Observations were made for visible growth of fungi. The highest dilution (lowest concentration) showing no visible growth was regarded as Minimum Inhibitory Concentration (MIC). Cells from the tubes showing no growth were sub cultured on SDA plates and incubated at 37^0c for 24 hours to determine if the inhibition was irreversible was permanent.

4. Result and Discusion

The study tested the antifugal activity of aqueous and organic extract and their respective dilutions from a medicinal plant (*Murraya koenigii*) against four fungi species namely*; Aspergillus niger, Penicillium camemberti, Candida albicans, Penicillium funiculosum.* This medicinal plant was chosen based on traditional usage and previous studies that have demonstrated antifungal properties using different kinds of extracts (Guo *et al*., 1997; Wilson *et al*., 1997; Zhu *et al*., 2005). Of the three extracts evaluated from the plant, all exhibited invitro antifungal activity with different inhibition values. According to the results obtained from the extraction of ethanol, distilled water and hot water, ethanol has a greater inhibitory value. In this regard, organic extract proved to have more activity against the various fungi species used than aqueous extracts. Thus it can be concluded that organic extract was more efficient in the extraction of water soluble biomolecules with antifungal activity. The aqueous extracts that demonstrated the least activity against the fungi species was cold water extract, this could be explained by the fact that when plant materials are ground and placed in cold water, some phenolases and hydrolases are released and could have modulating effects on the activity of the compounds in the extracts. It could also be due to incomplete extraction of the active components (El- Mahnood *et al*., 2008). These results are in accordance with Pinelo *et al*., (2004), who suggested that the chemical characteristics of the solvent, the method used during extraction process and diverse structural and compositional aspects of the natural products result in each material solvent system showing distinct behavior. Differences in polarity among various solvent have been reported to account for the differences in solubility of active plant properties, hence variation in the degree of activity (Itako *et al*., 2008). Comparison of the growth inhibition of various extracts and their respective dilutions shows a strong dependent effect on extract concentrations. These results revealed that antifungal activity of various extracts was enhanced by increasing the concentration of the extracts, thus the inhibition activity of the extracts was concentration dependent. This finding is in agreement with the report of Banso *et al*., (1999), who also observed that higher concentrations of substances showed more growth inhibition. In addition, the antifungal activity of plant extracts might not be due to the action of a single active compound, but the synergistic effect of several compounds that are in minor proportion in a plant (Davicino *et al*.,

2007). Therefore these data indicate that the appropriate extract concentration to show a specific effect depends on the plant used and the nature of the extract. Presence of constituents like flavonoids and tannins in the extracts, are likely to be responsible for the antimicrobial activity. This result indicates the potential usefulness of *Murraya koenigii* in the treatment of various pathogenic diseases.

5. Conclusion

The results obtained from this work showed that various extracts of *Murraya koenigii* (curry leaf) exhibited antifungal activity against *Candida albicans, Penicillium cumberti, Aspergillus niger, Aureobasidium pullulans.* In particular, organic extracts of *Murraya koengii* offer effective bioactive compounds for growth inhibition of the fungi, even at low concentrations it showed antifungal activity. The plant is therefore an ideal candidate in the search for new bioactive phyto compounds.

Further studies aimed at isolation and purification of active phyto constituents should be carried out.

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Table 1. Nutritional Value per 100g of Curry Leaves

Nutrition	Value per 100g
Moisture	63g
Fat	1g
Fiber	6g
Energy	108kcal
Phosphorus	57mg
Magnesium	44mg
Manganese	0.15mg
Chromium	0.006mg
Thiamine	0.08mg
Niacin	2.3mg
Vitamin C	4mg
Protein	6.1g
Minerals	4g
Carbohydrates	18g
Calcium	830mg
Iron	0.93mg
Copper	0.1mg
Zinc	0.2mg
Vitamin A	7560Ng
Riboflavin	0.21mg
Folic Acid	93.8mg

This table is according to specie board of India, the ministry of commerce and Industry, Government of India.

Table 2. Antifungal Activity of Plant Extracts on Test Organisms

S = Susceptible
R = Resistant
CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

Organism	Plant extract	20%	30%	40%	Standard
Candida albicans	CAE	S	S	S	S
	HAE	S	S	S	S
	EE	S	S	S	S
Penicillium cumberti	CAE	S	S	S	S
	HAE	S	S	S	S
	EE	S	S	S	S
Aureobasidium pullulans	CAE	R	S	S	S
	HAE	R	R	S	S
	EE	R	S	S	S
Aspergillus niger	CAE	R	S	S	S
	HAE	S	S	S	S
	EE	R	S	S	S

Table 3: Showing Diameters of Inhibition Zones (Rep 1)

CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

Organism	Plant extract	Diameter of borer(mm)	Zones of inhibition (mm)			
			20%	30%	40%	standard
Candida albicans	CAE	9	1	2	3	20
	HAE	9	2	5	6	21
	EE	9	1	2	3	23
Penicillium funiculosum	CAE	9	1	3	4	20
	HAE	9	4	5	6	21
	EE	9	3	4	6	23
Penicillium Camemberti	CAE	9	-	2	3	20
	HAE	9	-	-	7	21
	EE	9	-	6	7	23
Aspergillus niger	CAE	9	-	2	3	20
	HAE	9	2	3	4	21
	EE	9	-	6	11	23

Table 4: Showing Diameter of Inhibition zones Rep 1

CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

ORGANISM	PLANT EXTRACT	DIAMETER BORER (MM)	ZONES OF INHIBITION (MM)			
			20%	30%	40%	Standard
Candida albicans	CAE	9	1	2	3	20
	HAE	9	2	5	6	21
	EE	9	1	2	3	23
Penicillium funiculosum	CAE	9	1	3	4	20
	HAE	9	4	5	6	21
	EE	9	3	4	6	23
Penicillium camemberti	CAE	9	-	2	3	20
	HAE	9	-	-	7	21
	EE	9	-	6	7	23
Aspergillus niger	CAE	9	-	2	3	20
	HAE	9	2	3	4	21
	EE	9	-	6	11	23

Table 5: Showing Diameter of Inhibition Zones Rep 2

CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

Organism	Plant Extract	Diameter Borer(mm)	Zones of Inhibition (mm)			
			20%	30%	40%	Standard
Candida albicans	CAE	9	2	3	4	20
	HAE	9	1	6	7	21
	EE	9	2	3	5	23
Penicillium funiculosum	CAE	9	2	3	4	20
	HAE	9	5	6	8	21
	EE	9	4	5	8	23
Penicillium camemberti	CAE	9	-	2	3	20
	HAE	9	-	4	7	21
	EE	9	-	7	9	23
Aspergillus niger	CAE	9	1	2	3	20
	HAE	9	3	4	6	21
	EE	9	3	8	15	23

Table 6: Showing Diameter of Inhibition Zones REP3

CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

Organism	Plant Extract	Diameter Borer(mm)	Zones of Inhibition (MM)			
			20%	30%	40%	Standard
Candida albicans	CAE	9	1	4	5	20
	HAE	9	3	7	8	21
	EE	9	1	4	6	23
Penicillium funiculosum	CAE	9	3	5	6	20
	HAE	9	5	6	9	21
	EE	9	6	8	15	23
Penicillium camemberti	CAE	9	-	3	4	20
	HAE	9	-	6	6	21
	EE	9	-	8	10	23
Aspergillus niger	CAE	9	1	3	4	20
	HAE	9	5	6	9	21
	EE	9	3	9	13	23

Table 7: Showing mean of triplicates

CAE = Cold Aqueous Extract
HAE = Hot Aqueous Extract
EE = Ethanol Extract

Organism Plant Extract	Plant Extract	Diameter borer(mm)	Zones of Inhibition (mm)			
			20%	30%	40%	Standard
Candida albicans	CAE	9	1.3	3	4	20
	HAE	9	2	6	7	21
	EE	9	1.3	3	4.7	23
Penicillium funiculosum	CAE	9	2	3.7	4.7	20
	HAE	9	4.7	5.7	7.7	21
	EE	9	4.3	5.7	6.3	23
Penicillium Camemberti	CAE	9	0	2.3	3.3	20
	HAE	9	0	3	6.7	21
	EE	9	0	7	8.7	23
Aspergillus niger	CAE	9	0.7	2.3	3.3	20
	HAE	9	3.3	4.3	5.7	21
	EE	9	2	7.7	13	23

Plate 1. Nutrient Broth

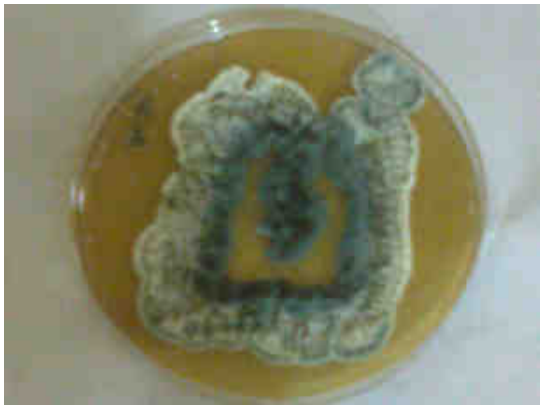

Plate 2: *Penicillium funiculosum*

Plate 3: *Penicillium camemberti*

Plate 4: ***Candida albicans***

Plate 5: ***Aspergillus niger***

Plate 6: Showing antifungal activity

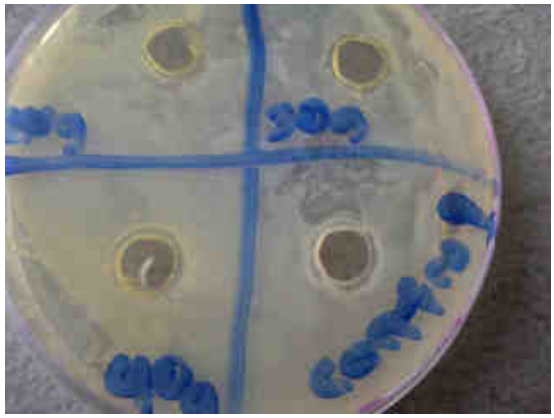

Plate 7: Showing antifungal activity

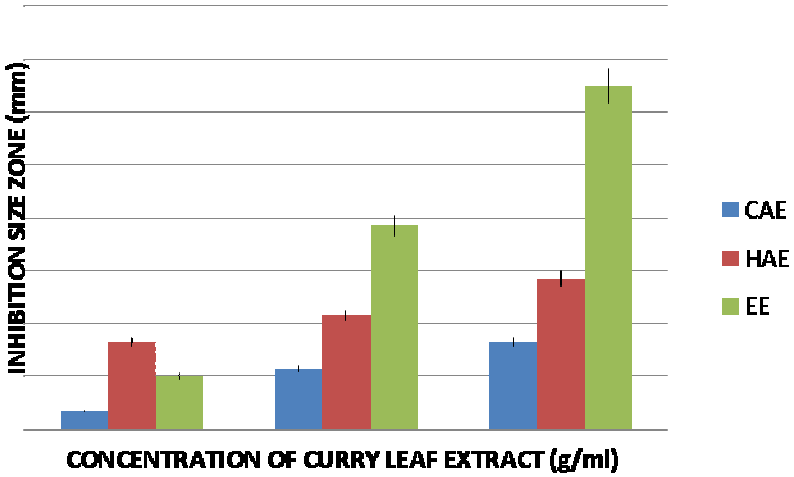

Figure 1. Growth inhibition of *Aspergillus niger* by leaf extracts of *Murrava koenaii*

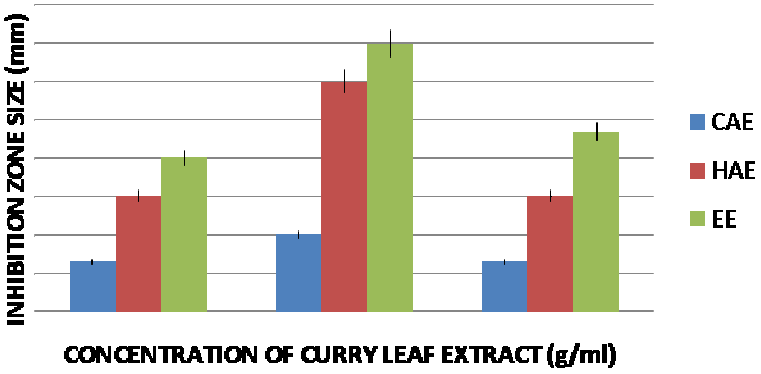

Figure 2. Growth inhibition of *Candida albicans* by leaf extracts of *Murrava koenaii*

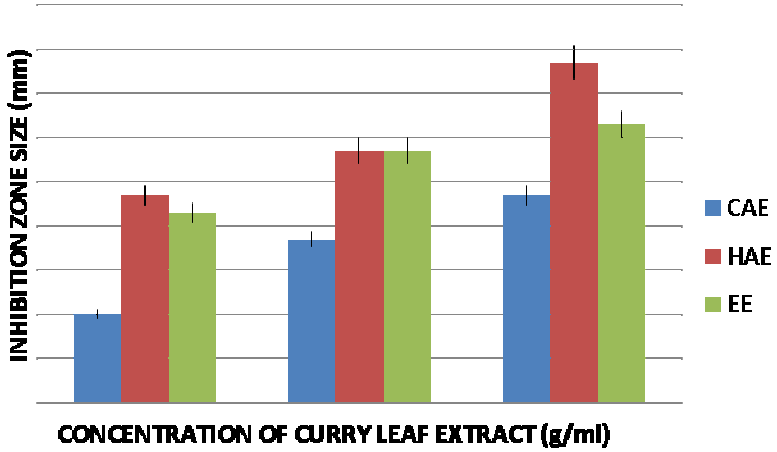

Figure 3. Growth inhibition of *Penicillium camemberti* by leaf extracts of *Murraya koengii*

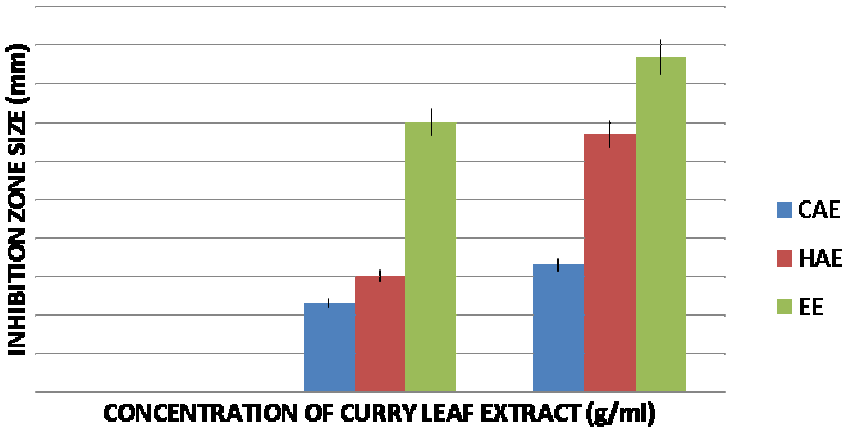

Figure 4. Growth inhibition of *Penicillium funiculosum* by leaf extracts of *Murraya koengii*

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